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cDNA sequence of the β_2-subunit of human liver alcohol dehydrogenase

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A cDNA library of mRNA from a human liver expressing the β_2-subunit of alcohol dehydrogenase was constructed in λgt11. One clone coding for 352 of a total of 374 amino acid residues of the β_2-subunit was isolated. The sequence differed from that of the β_1-subunit at one nucleotide position resulting in an Arg/His exchange at position 47 of the peptide chain, in agreement with data from protein sequence analysis [(1984) FEBS Lett. 173, 360–366].

Alcohol dehydrogenase; cDNA; NAD^+ binding site; Amino acid substitution; (Human liver)

1. INTRODUCTION

Human alcohol dehydrogenase (ADH; alcohol:NAD^+ oxidoreductase, EC 1.1.1.1) is a polymorphic enzyme that can be divided into three classes, I, II and III, differing in their catalytic and electrophoretic properties [1]. The class I isoenzymes consist of homo- and heterodimers of α-, β- and γ-subunits which are coded for by three gene loci, ADH_1, ADH_2 and ADH_3 [2]. Genetic alleles occur at the ADH_2 locus coding for β_1-, β_2- and β_3-subunits [2,3], and at the ADH_3 locus which codes for γ_1- and γ_2-subunits [2]. The catalytic properties of isoenzymes containing the β_2- and β_3-subunits differ significantly from those of the other isoenzymes. The specific activity is considerably higher and the pH optimum is shifted by at least two pH units toward more acidic values [4–6]. Protein sequence analysis of the three β-subunits indicated single amino acid exchanges. The Arg residues at position 47 and 369 of the β_1-subunit are replaced by His-47 in the β_2-subunit [7,8] and by Cys-369 in the β_3-subunit [9]. The Arg residues at both positions are involved in the binding of the coenzyme, and the substitutions have been implicated with the observed changes in the catalytic properties [7–9]. Recently, however, the α-subunit which does not share the altered catalytic properties of the β_2-chain was found to contain Gly at position 47 [10], suggesting that, in addition to the exchange at position 47, further differences might exist between the β_1- and β_2-subunits. To date structural data for the β_2-subunit are available only from partial amino acid sequence analysis. We, therefore, constructed a cDNA library from a human liver expressing the β_2-subunit. A cDNA coding for His at position 47 was isolated and sequenced. No additional amino acid exchange was detectable.

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2. MATERIALS AND METHODS

Tissue samples and mRNA obtained from the livers of organ donors were the kind gift of Professor Urs A. Meyer, University of Basle, Switzerland.

Livers were screened for the β_2-phenotype by taking advantage of the shift in the pH optimum caused by the β_2-subunit [4].

cDNA was synthesized by the RNase H method [11] using a commercially available kit (Amersham, Little Chalfont, England). The cDNA was ligated into *Eco*RI digested and dephosphorylated λgt11 DNA (Promega Biotech, Madison, WI) using synthetic *Eco*RI linkers (Pharmacia, Uppsala, Sweden) [12]. The recombinant λ DNA was packaged in vitro

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in the presence of extracts of *E. coli* BHB 2688 and BHB 2690 [13], and the phages were plated on *E. coli* Y1090 hsd R^- hsd M^+ (Promega Biotech).

The library was screened for ADH sequences by the plaque-hybridisation method [14]. A cDNA encoding approx. 1 kb of the human β_1-chain was radiolabeled by nick-translation [13], and hybridisation conditions (0.3 M NaCl, 0.03 M sodium citrate, 65°C) were chosen so as to give positive signals with all cDNAs coding for class I subunits.

Preparation of recombinant λ DNA was carried out by centrifugation in a CsCl step gradient [13].

cDNA coding for the β_2-subunit was detected by blotting the recombinant λ DNA on nitrocellulose membranes (Bio-Rad, Richmond, CA) followed by hybridisation with a β_2-specific oligonucleotide [15].

Insert cDNA was digested with different restriction enzymes (Boehringer, Mannheim, FRG) as indicated in fig.1. The generated fragments were subcloned into M13mp18/19 vectors [16] and sequenced using a modification of the dideoxy chain termination method [17].

3. RESULTS AND DISCUSSION

Among the 16 livers screened for the ADH phenotype, one liver obtained from a male Caucasian individual exhibited higher ethanol oxidizing activity at pH 8.5 than at 10.5, indicating the presence of the β_2-subunit. A cDNA library was prepared from 2 μg poly(A)$^+$ mRNA of this liver. Approx. 3×10^4 independent recombinant clones were obtained and, without amplification of the library, screened for ADH specific sequences. 12 clones gave a positive signal and were isolated by repeated plating. One of the clones, λADH 6, hybridized with the β_2-specific oligonucleotide and was subjected to sequence analysis. The sequence strategy is shown in fig.1. The cDNA extends from nucleotide position −38 to 1060, where A of the ATG start codon is base 1, and thus codes for amino acids 1–352 of the 374 residues of the protein chain. Compared to the nucleotide sequence of the β_1-subunit reported by Ikuta et al. [18] and Yokoyama et al. [19], and to the exons of the genomic β_1-sequence published by Duester et al. [20], the present sequence differs by only one nucleotide. It concerns the amino acid at position 47, where the present sequence has the triplet CAC coding for histidine instead of CGC coding for Arg in the β_1-subunit, in agreement with earlier protein sequence data [7,8]. Two additional differences were noted in comparison with the cDNA sequence reported by Heden et al. [21]. Their sequence shows an additional C in the non-translated

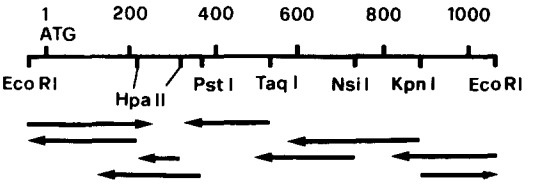

Fig.1. Sequencing strategy for the β_2-alcohol dehydrogenase cDNA.

5′-region at position −34 in their notation, which is not present in our sequence and in the genomic sequence reported by Duester et al. [20]. The second difference concerns the triplet coding for Ile-220, the third position of which is C in the sequence of Heden et al. [21], whereas in our sequence, as well as in the β_1 cDNA sequences reported by others [18,19] this position is occupied by T.

The present cDNA sequence terminates after 1098 nucleotides and lacks 65 nucleotides of the coding region as well as the complete 3′-noncoding part. The termination occurs at a position which in the cDNA coding for the β_1-subunit is followed by a cluster of adenosine nucleotides. Assuming a similar accumulation of A in the mRNA encoding the β_2-subunit, this region has probably served as a hybridisation target for the oligo-T primer during the synthesis of the first cDNA strand. The C-terminal part of the β_2-subunit which is not coded for by the present cDNA comprises 3 short tryptic peptides. A previous study has revealed no difference between the amino acid composition of these peptides [8] and the composition of the corresponding fragments derived from the β_1-subunit [22]. The present findings in conjunction with the data from the protein chemical studies are thus in full agreement with a single amino acid exchange between the β_1- and β_2-subunits.

Sequence analysis of all the subunits of human class I ADH has revealed a high degree of homology to the EE isoenzyme from horse liver. Despite the lack of crystallographic data for the human enzymes, it has been possible by use of computer graphics to construct a three-dimensional model of human alcohol dehydrogenase subunits based on the known structure of the horse enzyme [23]. The model accommodates all differences between the horse enzyme and the human isoenzymes in a highly conserved three-

dimensional structure and allows one to correlate structural differences between subunits with the catalytic properties of the corresponding isoenzymes. Especially, the model suggests that the residues involved in coenzyme binding are identical in the α-, β_1- and β_2-subunits with the exception of residue 47 which is Gly in the α-, Arg in the β_1- and His in the β_2-subunit, respectively. Since the rate-limiting step in alcohol oxidation is the dissociation of NADH [5,24], these differences should be reflected in the catalytic properties of the isoenzymes. Following the computer model, both Arg in the β_1-subunit and His in the β_2-subunit can form hydrogen and ionic bonds to the pyrophosphate of the coenzyme. Due to the lower pK_a of the imidazole relative to the guanidinium group, ionic forces should be lower in the β_2-subunit. The observed differences between the $\beta_1\beta_1$ and $\beta_2\beta_2$ isoenzymes – higher specific activity and lower pH optimum of the $\beta_2\beta_2$ isoenzyme [5] – can thus be related to the exchange at position 47 [7,8,23]. In the α-subunit Gly-47 neither forms a hydrogen bond to the coenzyme nor does it contribute to the binding via electrostatic forces [23]. NADH, therefore, should be bound even less tightly in the α- than in the β_2-subunit, resulting in a further increase in the specific activity and change in the pH optimum. Contrary to the prediction, however, the specific activity, K_m for the coenzyme and pH optimum of the $\alpha\alpha$ isoenzyme resemble much more the corresponding values for the $\beta_1\beta_1$ than for the $\beta_2\beta_2$ isoenzyme [5]. Since this study has not revealed any other difference between the β_1- and β_2-subunits than the Arg/His exchange at position 47 and, on the other hand, the α- and β_1-subunits differ by 24 residues, it must be assumed that residues other than the glycine at position 47 govern the rate and pH dependence of NADH dissociation in the α-subunit. This question may eventually be answered when crystals of the human ADH isoenzymes become available.

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